

Please amend the claims as shown in the List of Claims as follows.

List of Claims

1. (Currently amended) A transgenic *Dunaliella Salina* bioreactor comprising:  
*Dunaliella Salina* as host,  
transformed to provide transgenic *Dunaliella Salina* with a foreign target gene selected from the group consisting of tumor necrosis factor (TNF) and hepatitis B surface antigen (HBsAg); and a selectable marker selected from the group consisting of the *aadA* gene encoding for spectinomycin or streptomycin resistance, and BAR gene encoding for herbicide phosphinothricin (PPT) resistance;  
culturing the transgenic *Dunaliella Salina* in a culture medium selected from the group consisting of Mclachlan culture fluid and a combination culture fluid A comprising 5mM NH<sub>4</sub>Cl and 5mM NaNO<sub>3</sub> and culture fluid B containing 10mM NaNO<sub>3</sub> to express the foreign target gene.
2. (cancelled)
3. (cancelled)
4. (cancelled)
5. (cancelled)
6. (Currently amended) A method for preparing a transgenic *Dunaliella Salina* bioreactor, comprising the following steps:
  - (a) transforming the cells of *Dunaliella Salina* to provide transgenic *Dunaliella Salina* by introducing into the cells of *Dunaliella Salina* an expression vector comprising a foreign target gene selected from the group consisting tumor necrosis factor gene and hepatitis surface antigen gene; together with a selectable marker selected from the group consisting of *aadA* gene encoding for spectinomycin or

streptomycin resistance, BAR gene encoding for herbicide phosphinothricin (PPT) resistance; and

- (b) culturing the transgenic Dunaliella Salina in a liquid medium selected from the group consisting of Mclachlan culture fluid, and a combination culture fluid A comprising 5mM NH<sub>4</sub>Cl and 5mM NaNO<sub>3</sub> and culture fluid B containing 10mM NaNO<sub>3</sub>; and
  - (c) screening the cells of transgenic Dunaliella Salina for expression of the selectable marker showing transformation and expression of the foreign target gene.
- 7. (Previously presented) A method as claimed in step (a) of claim 6, wherein the cells of Dunaliella Salina were transformed by a method selected from the group consisting of a physical and a chemical method.
  - 8. (cancelled)
  - 9. (Previously presented) The method according to claim 7, wherein said physical method is selected from the group consisting of electroporation, use of a gene gun and wherein said chemical method is selected from the group consisting of PEG-mediated transformation.
  - 10. (cancelled)
  - 11. (cancelled)
  - 12. (Currently amended) The method according to claim 6 wherein the cells of transformation is by construction of a Dunaliella Salina are transformed by introducing an expression vector containing a foreign target gene selected from the group consisting of a fragment of TNF gene and HBsAg gene, and transforming the cells of Dunaliella Salina with the Dunaliella Salina expression vector
  - 13. (Currently amended) The method according to claim 12, wherein the Dunaliella Salina expression vector is constructed by the steps:

- (a) cloning the *Dunaliella Salina* chloroplast *atpA* 5' promoter sequence and *rbcl* 3' terminator sequence;
  - (b) constructing a plasmid pUC19-TNF containing a cDNA fragment of TNF;
  - (c) digesting the plasmid pUC19-TNF with a restriction endonuclease to provide an intermediate vector pSK-*atpK*-TNF for an expression cassette of the TNF cDNA fragment;
  - (d) Constructing a vector p64C containing a cloned homologous fragment of the *Dunaliella Salina* chloroplast gene, *clpP-trnI-petB*, together with a *chlL* gene encoding the 5' promoter and the 3' terminator of the *chlL* gene;
  - (e) Inserting the expression cassette of TNF cDNA fragment into the p64C vector to provide an intermediate chloroplast expression vector, p64C-*atpX*-TNF;
  - (f) Locating the expression cassette of TNF cDNA fragment downstream of the *chlL* 5' promoter;
  - (g) Constructing an expression cassette of *aadA* gene encoding for spectinomycin resistance; and
  - (h) Constructing a chloroplast expression vector comprising the expression cassette of TNF cDNA fragment and the expression cassette of *aadA* gene.
14. (Currently amended) The method according to claim 12 wherein the target gene is HBsAg comprising the steps:
- (a) amplifying a SS1 fusion gene ~~fragment~~ comprising the fusion of a gene ~~fragment~~ encoding amino acid residues 1-226 of HBsAg and a PreS1 gene ~~fragment~~ encoding amino acid residues 20-48 of PreS1 of hepatitis B viral gene and ligating the fusion gene at Sal I/SphI site ~~to obtain SS1 fusion gene~~;

- (b) Constructing a plasmid, pUC18-CtxB-SS1, comprising CtxB gene of cholera toxin B subunit gene and the SS1 fusion gene;
- (c) cloning the 5' promoter and the T-Nos terminator of a heat shock protein, Hsp70B of *Dunaliella Salina* to obtain plasmid pSP72-Hsp-Nos;
- (d) Ligating the pUC18-CtxB-SS1 into plasmid pSP72-Hsp-Nos between the 5' promoter and the T-Nos terminator sequences to obtain an expression cassette of CtxB-SS1;
- (e) Constructing an expression cassette expressing nitrate reductase (Nit1) and inserting it into an expression cassette comprising MAR1 and MAR2 of the matrix attachment regions of a *Dunaliella Salina* expression vector PCAMBRIA-OS1644 in the same orientation;
- (f) Constructing a BAR expression cassette expressing BAR encoding for PPT resistance;
- (g) Integrating the expression cassettes, Nit1, CtxB-SS1 and BAR to an active transcription region of *Dunaliella Salina* chromosome.